Sample Analysis Report

Number 40

Cary 50 Microvolume Applications in Biochemistry and Molecular Biology; DNA/RNA, Proteins and Kinetics

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Introduction:

The Very small beam geometry, 1.5 nm bandwidth, and very high intensity flash source of the Cary 50, coupled with a long pathlength Czerny-Turner monochromator design and ultra-fast scanning capability, make the Cary 50 a uniquely powerful instrument. The ease of use is further enhanced due to the room-light immunity of the system, which removes the need to close the sample compartment when taking a reading. The standard Cary 50 microcell holder provides sufficient precision of alignment to easily interchange between microcell and conventional cuvettes. Adding the 18-position autochanger gives a fully automated capability to read up to 18 kinetic samples at precisely regulated temperatures between 20 and 60 degrees.

The Cary 50 uses a Xenon Flash source lamp, which is so durable it should never need replacement. The instrument requires no warm-up time, and the light beam, although intense during the sub-microsecond flash, is unlikely to photo-degrade sensitive samples as the average intensity is low, and the sample is only ever illuminated with monochromatic light.

The Hellma Traycell (figure 1) provides a simple and very precise solution to low-volume measurements, particularly when used in the Cary 50 due to the very small beam geometry. (Figure 2)

Figure 1

The Hellma TrayCell Accessory provides 1-5 microlitre sampling capability for the Cary 50

Figure 2 The Award winning Cary 50 is an exceptionally versatile instrument

Instrumentation and Conditions:

The Cary 50 was validated as completely within specification using the inbuilt automatic validate software module. The TrayCell accessory was placed in the quick-release mount high precision cell holder (Varian part No 0210167200) and aligned for height and pitch to give optimal light throughput. (See Installation and Alignment guide) *Once aligned for the instrument no further adjustments are required*.

A range of samples was supplied for analysis. These included DNA from various sources, Proteins at a range of concentrations and an enzyme kinetic reaction study.

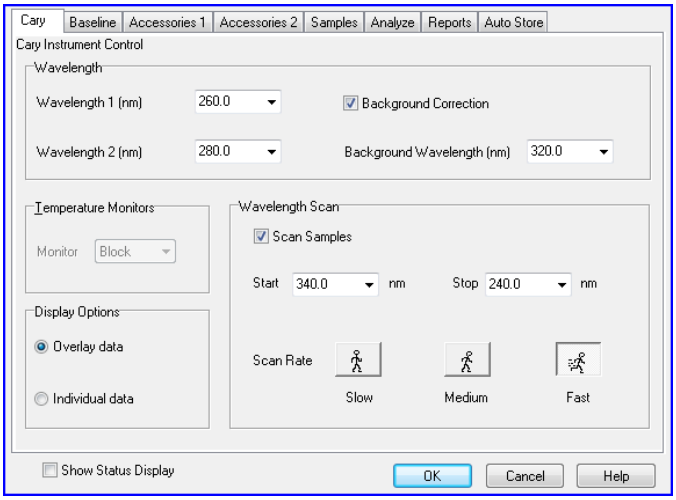
Figure 3

The standard cell holders for the Cary 50 cover the range from 1ul to 100ml and offer temperature control in 1cm cells from 0-100°C *Multicell holders and fiberoptics are also available*

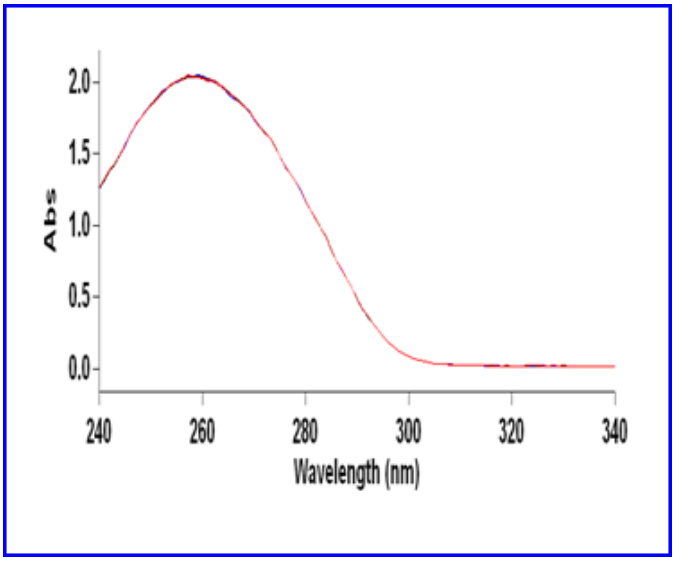
Results:

DNA was provided in the form of a concentrated PCR sample. This was read directly in 4ul in the TrayCell, and then diluted 100 fold and re-read using a standard 1 cm path 400 ul Cuvette. Setup using the onboard RNA/DNA software module as;-

***Results Figure 1**. Setup of DNA/RNA Software*



***Results Plot 1** Scan of 4ul Concentrated DNA solution in Traycell*

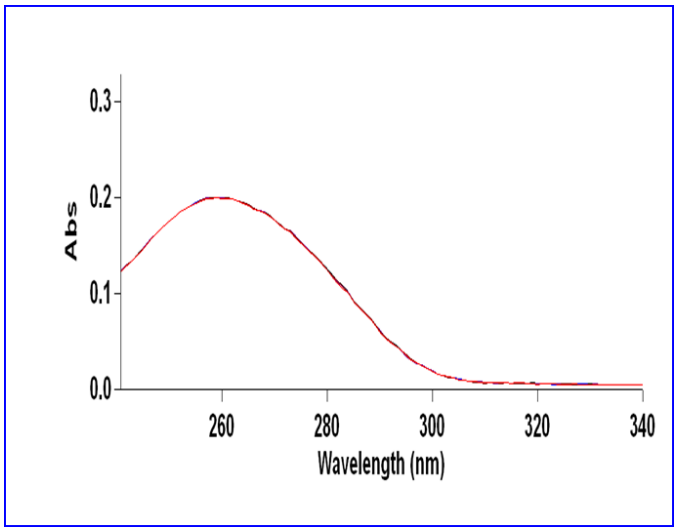


Analysis

Collection time 07/07/2005 11:48:37

Ratio	Protein µg/ml	Nucleic Acid µg/ml
1.7474	263.4789	1224.879
1.7586	252.9489	1229.443
1.7469	263.5379	1222.926
1.7509	260.2943	1226.499

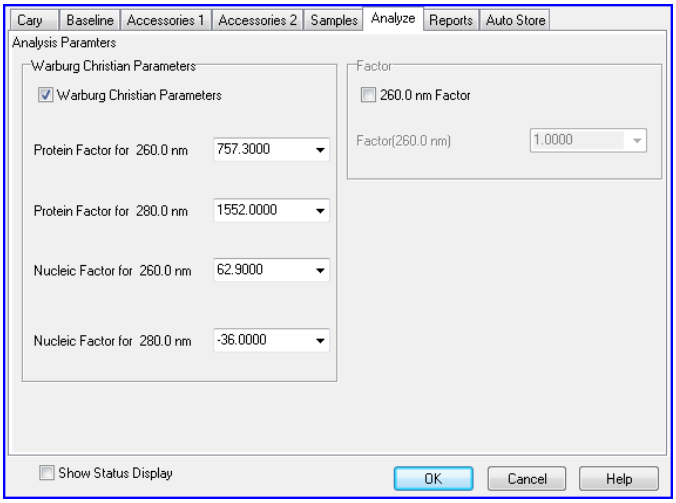
***Results Plot 2** Diluted DNA solution in 400ul 1 cm path cell*



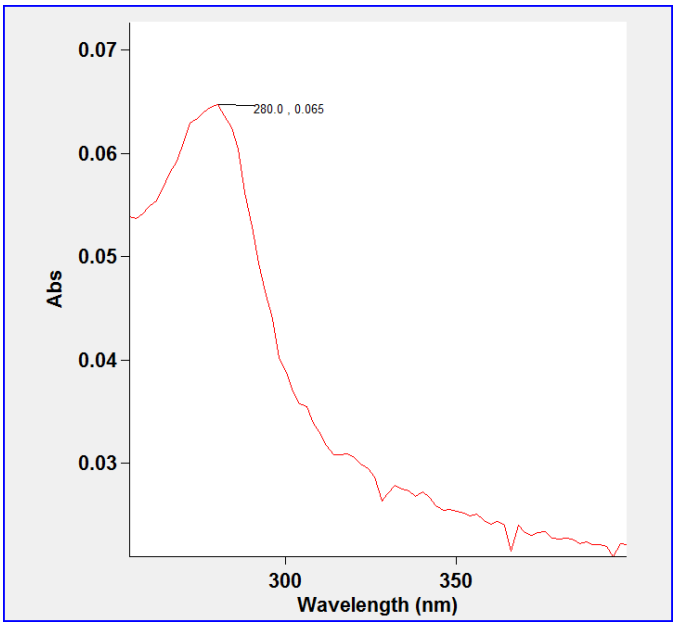
Ratio	Protein µg/ml	Nucleic Acid µg/ml
1.6270	38.2096	11.7958
1.6376	36.9177	11.7696
1.6321	37.6365	11.7986
1.6340	37.3303	11.7697

Calculation of the DNA content of the sample measured in both the Microvolume cell and the standard 1 cm path cell gave results which were reproducible to within less than 0.5 % (re-reads of same sample) but the Microvolume result was shown to be just as precise and reproducible as the standard cell. The discrepancy between the calculated results is probably due to dilution error.

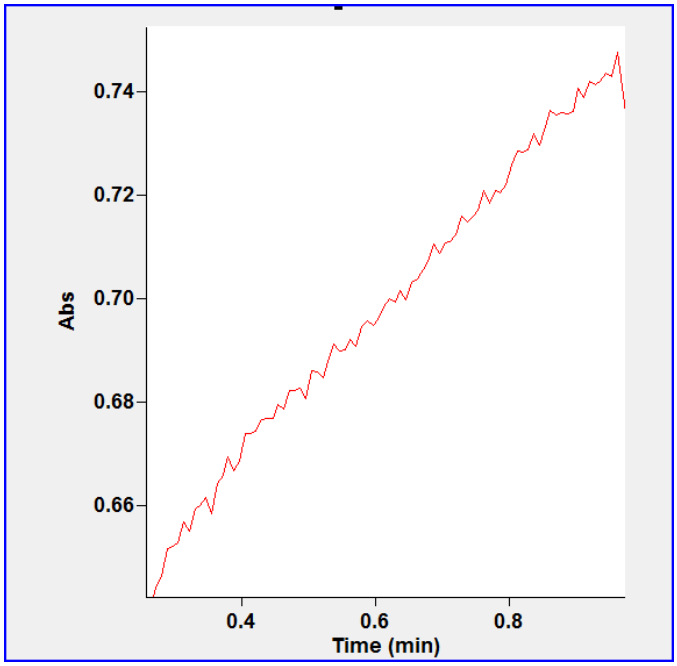
***Results Figure 2** Calculation screen parameters*



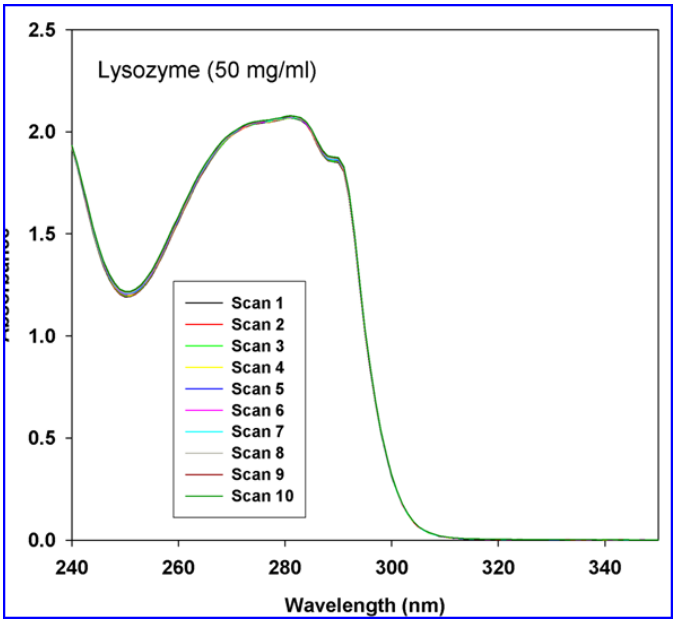
Results Plot 3 *Dilute Protein Sample in 1 ul in Traycell (data has not been smoothed)*



Results Plot 5 *Kinetic reaction in 4ul sample in TrayCell/Cary 50*



Results Plot 4 *50 mg/ml Protein (Lysosyme) in 1ul in TrayCell*



Scans repeated at 2 minute intervals to show the ability of the Traycell to prevent evaporative losses over the time-course of a kinetic reaction. The total variance over 30 minutes was less than 0.002 Abs at 2 Abs (280nm)

Point to point noise on the linear part of the curve (curve fit shown) is about 0.002A but total change of absorbance in 1 minute is .1A giving an overall signal to noise better than 50:1.

Discussion

The Cary 50 platform is able to deliver open beam baseline stability to better than 0.0005A. (Typical is 0.0002A) The fine focus of the Cary 50 beam allows very efficient coupling to either standard fiberoptics, (Up to 60%T (transmission)) Microvolume full pathlength cells (down to 20ul at 60%T) or to the Hellma TrayCell (30%T)

Because the energy loss is comparatively small (30%T = approx 0.5Abs) the Cary 50 can still deliver a good dynamic range (open beam 3.3 Abs, TrayCell to 2.6 Abs) and excellent signal over noise performance even with 1ul samples. The Cary 50 Illuminates the sample with1.5nm bandwidth monochromatic light and can scan 300nm in a second. These characteristics provide ideal conditions for the easy to use TrayCell Microvolume cell to offer far higher performance, resolution, stability and sensitivity than conventional compact microvolume spectrophotometers.

For further information, contact your local Varian Sales Office. 01865 291500